Investigating the purpose of prelamin A processing

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Lmna yields two major protein products in somatic cells, lamin C and prelamin A. Mature lamin A is produced from prelamin A by four posttranslational processing steps—farnesylation of a carboxyl-terminal cysteine, release of the last three amino acids of the protein, methylation of the farnesylcysteine, and the endoproteolytic release of the carboxyl-terminal 15 amino acids of the protein (including the farnesylcysteine methyl ester). Although the posttranslational processing of prelamin A has been conserved in vertebrate evolution, its physiologic significance remains unclear. Here we review recent studies in which we investigated prelamin A processing with *Lmna* knock-in mice that produce exclusively prelamin A (*Lmna*^{PLAO}), mature lamin A (*Lmna*^{LAO}) or nonfarnesylated prelamin A (*Lmna*^{nPLAO}). We found that the synthesis of lamin C is dispensable in laboratory mice, that the direct production of mature lamin A (completely bypassing all prelamin A processing) causes no discernable pathology in mice, and that exclusive production of nonfarnesylated prelamin A leads to cardiomyopathy.

Introduction

The nuclear lamina is an intermediate filament meshwork that lies beneath the inner nuclear membrane.¹ Lamin A and lamin C, two key structural proteins of the nuclear lamina, are produced by alternative splicing of *Lmna* and are commonly known as A-type lamins.² Along with the B-type lamins, lamins A and C oligomerize to form a filamentous lattice that provides structural support for the nucleus.^{1,3}

Both nuclear lamins also interact with a number of proteins in the heterochromatin and nuclear membrane.⁴

Lmna knockout mice (*Lmna*^{-/-}) clearly demonstrated the importance of A-type lamins in mammals.⁵ *Lmna*^{-/-} mice appear normal at birth but grow slowly and die by 5–6 weeks of age, with histopathologic evidence of muscular dystrophy.⁵ Heterozygous knockout mice (*Lmna*^{+/-}) develop late-onset dilated cardiomyopathy and conduction system disease.⁶ The absence of lamins A and C disrupts the structural integrity of the nuclear envelope, resulting in grossly misshapen cell nuclei⁵ and defective nuclear mechanics.⁷

The first 566 amino acids of lamins A and C are identical, but their carboxyl-terminal domains diverge.^{2,8-10} Lamin C (572 amino acids) terminates with exon 10 sequences and contains six carboxyl-terminal amino acids not found in prelamin A. Prelamin A (664 amino acids) contains 98 additional amino acid sequences encoded by exons 11 and 12 not found in lamin C, and terminates with a carboxyl-terminal *CaaX* motif. Prelamin A is converted to mature lamin A by four enzymatic posttranslational processing steps—farnesylation of a cysteine located four amino acids from the carboxyl terminus of the protein (carried out by protein farnesyltransferase), endoproteolytic cleavage of the last three amino acids of the protein (a redundant activity of RCE1 and ZMPSTE24), carboxyl methylation of the newly exposed farnesylcysteine (by ICMT), and endoproteolytic release of the last 15 amino acids of the protein, including the farnesylcysteine methyl ester (by ZMPSTE24).¹¹⁻¹⁹ The final cleavage step releases mature lamin A, which contains 646 amino acids.

Key words: prelamin A, progeria, restrictive dermopathy, protein farnesylation, cardiomyopathy

Abbreviations: HGPS, hutchinson-gilford progeria syndrome; RD, restrictive dermopathy; FTI, farnesyltransferase inhibitor

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Disruption of prelamin A processing has been linked to progeroid disorders in humans and mice. Hutchinson-Gilford progeria syndrome (HGPS) is caused by heterozygosity for *LMNA* point mutations that alter mRNA splicing and results in a 50-amino acid internal deletion in prelamin A.²⁰ This deletion does not affect prelamin A's *CaaX* motif but removes the site for the final cleavage by ZMPSTE24, preventing normal lamin A biogenesis and causing an accumulation of a shortened prelamin A (often called progerin) that retains a carboxyl-terminal farnesylcysteine methyl ester.^{21,22}

In humans, a deficiency in ZMPSTE24 causes a perinatal-lethal progeroid syndrome, restrictive dermopathy (RD). Without ZMPSTE24, the final processing step that releases mature lamin A cannot occur, resulting in an accumulation of a farnesylated and methylated prelamin A. Unlike progerin, the prelamin A in RD does not contain the internal deletion but retains the carboxyl-terminal 15-amino acid segment that is normally clipped off in the formation of mature lamin A.

The accumulation of farnesylated progerin (in HGPS) and noncleaved farnesylated prelamin A (in RD) disrupts the structural integrity of the nuclear lamina. HGPS fibroblasts (both mouse and human) as well as ZMPSTE24-deficient fibroblasts (both human and mouse) contain dramatically misshapen cell nuclei, with a high frequency of blebs and folds.^{18-20,23}

Prenylation and Progeria

The fact that farnesylated progerin in HGPS and the farnesylated prelamin A in the setting of ZMPSTE24 deficiency cause misshapen nuclei and severe disease phenotypes is well established, but the mechanism by which they elicit disease is less clear. Several studies have suggested that the retention of the carboxyl-terminal farnesylcysteine methyl ester is a key factor in disease pathogenesis. The frequency of misshapen nuclei in HGPS and RD fibroblasts can be reduced with a protein farnesyltransferase inhibitor (FTI).²⁴⁻²⁸ Also, the progeria-like disease phenotypes in HGPS knock-in mice (*Lmna*^{HG/+} mice, expressing farnesylated progerin) and

Zmpste24-deficient mice (expressing noncleaved farnesylated prelamin A) could be ameliorated with an FTI.²⁹⁻³² However, recent studies by Yang et al.²² have suggested that the persistence of the farnesylcysteine may not be the entire story. They showed that knock-in mice expressing a *nonfarnesylated* version of progerin (*Lmna*^{nHG/+}) still developed progeria, although the severity of the disease was somewhat less than in the mice producing farnesylated progerin.²² The latter finding implied that the farnesyl lipid may not be the sole factor in disease pathogenesis, and that the 50-amino acid deletion may also be relevant.

The discovery of significant disease in the *Lmna*^{nHG/+} mice led Davies et al.³³ to hypothesize that the retention of the last 15 amino acids of prelamin A in *ZMPSTE24*-deficiency, and not merely the terminal farnesyl cysteine methyl ester, might be important in the pathogenesis of RD. To test this idea, they generated a *Lmna* knock-in allele that exclusively produced *nonfarnesylated* prelamin A. At the same time, a knock-in allele that produced exclusively *wild-type* prelamin A was generated.³³ The “nonfarnesylated prelamin A-only” allele (*Lmna*^{nPLAO}) and the “prelamin A-only” allele (*Lmna*^{PLAO}) were identical except that the cysteine in the *CaaX* motif of the *Lmna*^{nPLAO} allele was changed to serine, thereby abolishing protein prenylation. Introns 10 and 11 were removed from both alleles, abolishing lamin C synthesis. Western blots of tissue extracts and embryonic fibroblasts from homozygous “nonfarnesylated prelamin A-only” (*Lmna*^{nPLAO/nPLAO}) mice showed that the *Lmna*^{nPLAO} allele produced exclusively prelamin A; subsequent metabolic labeling studies showed that this prelamin A was not farnesylated. Western blots of tissue extracts from *Lmna*^{PLAO/PLAO} mice revealed the production of only mature lamin A (generated by the normal post-translational processing of wild-type prelamin A).³³

Davies and colleagues reasoned that if the disease phenotypes in RD were truly elicited by the retention of the last 15 amino acids of prelamin A (rather than the farnesyl lipid), then the phenotypes of *Lmna*^{nPLAO/nPLAO} mice would be expected to resemble those of *Zmpste24*^{-/-} mice.

This was not the case. Unlike *Zmpste24*^{-/-} mice, which gain weight slowly, develop micrognathia and osteolytic lesions in the bones, and die prematurely^{16,18,19} (phenotypes shared by humans with prelamin A-related progeroid syndromes^{20,34,35}), *Lmna*^{nPLAO/nPLAO} mice have no progeria phenotypes of any kind; they maintain virtually normal body weights and lack osteolytic lesions.³³ These findings suggest that—at least in the setting of full-length prelamin A—the farnesyl lipid is critical for the emergence of disease phenotypes.

Cardiomyopathy in *Lmna*^{nPLAO/nPLAO} Mice

Although the *Lmna*^{nPLAO/nPLAO} mice did not exhibit progeroid-like phenotypes, they were not phenotypically normal. Male *Lmna*^{nPLAO/nPLAO} mice died prematurely, with a median survival of 38.5 weeks. Female *Lmna*^{nPLAO/nPLAO} mice lived slightly longer but still had a markedly reduced median survival of 49.5 weeks.³³ Echocardiography revealed that *Lmna*^{nPLAO/nPLAO} mice had a dilated cardiomyopathy, with a reduced left ventricular ejection fraction and an increased end-systolic left ventricular chamber size. As is typical for cardiomyopathy,³⁶ the expression of myosin heavy chain alpha (*Myh6*) was reduced in the hearts of *Lmna*^{nPLAO/nPLAO} mice, and the expression of myosin heavy chain beta (*Myh7*) was increased.³³ *Lmna*^{PLAO/PLAO} mice displayed no signs of cardiomyopathy and exhibited normal survival.

The finding of cardiomyopathy in *Lmna*^{nPLAO/nPLAO} mice was not entirely unexpected, given that *LMNA* missense mutations in humans have been linked to dilated cardiomyopathy and muscular dystrophy.³⁷⁻³⁹ When some of these disease-associated mutations were introduced into mouse *Lmna*, they have resulted in cardiomyopathy and premature death.^{40,41}

There are two potential explanations for the cardiomyopathy in *Lmna*^{nPLAO/nPLAO} mice. One possibility is that nonfarnesylated prelamin A is a toxic molecule that leads to cardiac dysfunction. A toxic effect is quite plausible, especially in light of the observation that progeria disease phenotypes are positively correlated with the amount of farnesylated prelamin A.

When the levels of farnesylated prelamin A in mice are reduced by one-half in *Zmpste24*^{-/-} mice (by introducing a *Lmna* knockout allele or a lamin C-only allele), disease phenotypes are completely eliminated.^{18,42} Conversely, increasing farnesylated prelamin A levels in *Zmpste24*^{-/-} mice (by introducing the *Lmna*^{PLAO} allele) causes more severe disease.³³ The second potential explanation for cardiomyopathy in *Lmna*^{nPLAO/nPLAO} mice is that the non-farnesylated prelamin A is simply a less effective lamin than wild-type lamin A. In other words, nonfarnesylated prelamin A is a poor substitute for lamin A (or lamin C) and is functionally less active—rather than being toxic. Heterozygosity for *Lmna* deficiency in mice causes cardiomyopathy in mice;⁶ thus, it is quite plausible that a functionally hypoactive lamin could underlie the cardiomyopathy in *Lmna*^{nPLAO/nPLAO} mice.

To differentiate between these two possibilities (i.e., a toxic lamin vs. a hypoactive lamin), *Lmna*^{nPLAO/+} mice were intercrossed with *Lmna*^{+/-} mice to generate *Lmna*^{nPLAO/-} mice. *Lmna*^{nPLAO/-} mice produce exclusively nonfarnesylated prelamin A, but only half as much as *Lmna*^{nPLAO/nPLAO} mice. We reasoned that if nonfarnesylated prelamin A were toxic, *Lmna*^{nPLAO/-} mice would be protected from cardiomyopathy. On the other hand, if the cardiomyopathy in *Lmna*^{nPLAO/nPLAO} mice was due to a functionally hypoactive lamin, we would expect *Lmna*^{nPLAO/-} mice to have more severe disease. The outcome of this experiment was unequivocal; *Lmna*^{nPLAO/-} mice had greatly shortened survival (median survival, 14.6 weeks) compared with *Lmna*^{nPLAO/nPLAO} mice (median survival, 38.5 weeks) (Fig. 1). These genetic studies suggest that the cardiomyopathy in *Lmna*^{nPLAO/nPLAO} is caused by a functionally hypoactive lamin, not a toxic lamin.

The examination of a *Lmna* knock-in allele yielding the farnesylated form of progerin (*Lmna*^{HG}) led to the opposite conclusion. In that case, the mutant lamin was clearly toxic. Mice carrying a single copy of the mutant allele and one copy of the knockout allele (*Lmna*^{HG/-}) survived longer than mice carrying two copies of the mutant allele (*Lmna*^{HG/HG}).⁴³ Thus, we suggest that lamin A mutations can be divided into two categories—those

yielding a toxic lamin (e.g., farnesylated progerin in HGPS) and those yielding a functionally hypoactive lamin (e.g., nonfarnesylated prelamin A in cardiomyopathy).

Purpose of Prelamin A Processing

Several studies have suggested that prelamin A's hydrophobic lipid anchor is important for the targeting of the protein to the inner nuclear membrane.^{15,44–46} If this were the case in vivo, one could reasonably propose that nonfarnesylated prelamin A is a poorly functioning lamin protein because it cannot reach the nuclear rim. However, we found scant evidence for defective targeting of nonfarnesylated prelamin A. In embryonic fibroblasts, the amount of nonfarnesylated prelamin A at the nuclear rim was somewhat less than the amount of mature lamin A. However, the targeting of nonfarnesylated prelamin A to the nuclear rim in tissues of *Lmna*^{nPLAO/nPLAO} mice was perfectly normal, identical to that of mature lamin A in *Lmna*^{PLAO/PLAO} tissues.³³ Thus, the incorporation of prelamin A into the nuclear lamina does not appear to be dependent on protein farnesylation. These findings are consistent with several other recent reports. First, lamin C (which is never farnesylated) reaches the nuclear rim in the absence of lamin A.⁴²

Figure 1. Survival curves for *Lmna*^{+/+}, *Lmna*^{nPLAO/+} and *Lmna*^{nPLAO/-} mice. The survival time for male *Lmna*^{nPLAO/-} mice was shorter than for *Lmna*^{+/+} or *Lmna*^{nPLAO/+} mice ($n = 4$ for each group) ($p = 0.002$). Previous studies showed that male *Lmna*^{nPLAO/nPLAO} mice had an average survival time of 38.5 weeks (a milder phenotype than *Lmna*^{nPLAO/-} mice),³³ suggesting that nonfarnesylated prelamin A is a poorly functioning lamin.

Also, Lee et al.⁴⁷ showed that the nuclear rim localization of nonfarnesylated prelamin A in protein farnesyltransferase-deficient keratinocytes is indistinguishable from that of mature lamin A in wild-type keratinocytes.

To further explore the purpose of the multistep posttranslational processing pathway for prelamin A, Coffinier et al.⁴⁸ generated a knock-in *Lmna* allele (*Lmna*^{LAO}) that yields mature lamin A directly, bypassing prelamin A synthesis and processing. These “mature lamin A-only” mice were generated by introducing a stop codon into *Lmna* immediately after the codon for the last amino acid of mature lamin A.⁴⁸ *Lmna*^{LAO/PLAO} mice produced only mature lamin A (and no prelamin A or lamin C), and the levels of mature lamin A in these mice were identical to those in *Lmna*^{PLAO/PLAO} mice (where all of the mature lamin A is derived from prelamin A). We fully expected that the absence of prelamin A processing in *Lmna*^{LAO/PLAO} mice would be accompanied by significant pathology, particularly in view of the conservation of the prelamin A processing pathway in vertebrate evolution.^{45,49–51} To our surprise, this was not the case. *Lmna*^{LAO/PLAO} mice had normal body weights, normal survival, and lacked discernable disease phenotypes—even in mice >18 months of age.⁴⁸

Figure 2. For figure legend, see page 8.

Figure 2 (See previous page). The effects of disrupting A-type lamin synthesis and processing in laboratory mice. (A) Wild-type mice produce both lamin C and prelamin A. Prelamin A then undergoes four sequential posttranslational processing steps to generate mature lamin A. (B) *Lmna*^{-/-} mice produce neither lamin A nor lamin C. These mice have muscular dystrophy and die at 5–6 weeks of age.⁵ (C) *Lmna*^{LCO/LCO} mice produce only lamin C. These mice appear to be phenotypically normal.⁴² (D) *Lmna*^{PLAO/PLAO} mice do not produce lamin C. The prelamin A produced by these mice is efficiently converted to mature lamin A. These mice also appear to be normal.³³ (E) *Lmna*^{LAO/LAO} mice produce mature lamin A directly, bypassing prelamin A synthesis and processing. These mice make no lamin C. Like *Lmna*^{LCO/LCO} and *Lmna*^{PLAO/PLAO} mice, *Lmna*^{LAO/LAO} mice appear to be normal.⁴⁸ (F) *Lmna*^{nPLAO/nPLAO} produce exclusively nonfarnesylated prelamin A. Because of a cysteine-to-serine substitution in prelamin A's CaaX motif, the prelamin A cannot be farnesylated. These mice develop dilated cardiomyopathy and have reduced survival.³³ (G) *Zmpste24*-deficient mice produce both lamin C and prelamin A, but the final endoproteolytic step does not occur, resulting in the accumulation of farnesylated prelamin A. These mice display multiple progeria-like disease phenotypes.^{16,19} (H) In addition to making normal lamin C and prelamin A from one allele, *Lmna*^{HG/+} mice make a prelamin A with a 50-amino acid internal deletion (from the *Lmna*^{HG} allele). This deletion prevents the final, ZMPSTE24-mediated, endoproteolytic step from occurring and leads to the accumulation of a farnesylated and truncated form of prelamin A called progerin. Despite the presence of normal forms of lamin A and lamin C, these mice develop progeria-like disease phenotypes.^{28,29} (I) *Lmna*^{nHG/+} mice are identical to *Lmna*^{HG/+} mice except that the truncated prelamin A generated from the *Lmna*^{HG} allele cannot be farnesylated (due to a cysteine-to-serine substitution in the CaaX motif), leading to the accumulation of nonfarnesylated progerin. These mice also have progeria, though it is somewhat less severe than that found in *Lmna*^{HG/+} mice.²²

And quite remarkably, the mature lamin A in *Lmna*^{LAO/LAO} tissues was normally positioned at the nuclear rim, indistinguishable from the lamin A in the tissues of wild-type or *Lmna*^{PLAO/PLAO} mice. The only abnormality that we identified was in *Lmna*^{LAO/LAO} fibroblasts; those fibroblasts had more nuclear shape deformities than wild-type fibroblasts, and a greater percentage of the mature lamin A in *Lmna*^{LAO/LAO} fibroblasts seemed to be located in the nucleoplasm (as opposed to the nuclear rim).⁴⁸

Given that the prelamin A processing pathway is evolutionarily conserved, we believe that it is overwhelmingly likely that the pathway serves some important function. At this point, however, current evidence suggests that the prelamin A processing pathway is largely dispensable, at least in laboratory mice. It is entirely possible that a more subtle phenotype in the *Lmna*^{LAO/LAO} (or *Lmna*^{LCO/LCO}; see below) mice may not have been detectable in our initial studies, and that future studies very well may uncover a unique functional role for prelamin A processing in mammals.

Lamin A or Lamin C: Why Two Isoforms?

Both *Lmna*^{LAO/LAO} and *Lmna*^{PLAO/PLAO} mice appeared to be phenotypically normal, despite a complete absence of lamin C.^{33,48} Similarly, Fong et al.⁴² found that knock-in mice that produced exclusively lamin C and no lamin A (*Lmna*^{LCO/LCO}) were phenotypically normal. In contrast, mice lacking both lamin A and lamin C succumb to muscular dystrophy.⁵ These observations strongly imply that lamin A and lamin C are crucial in mammals, but

that they play largely redundant roles and that either isoform is sufficient to prevent disease. Once again, however, we believe that much remains to be learned, and that sooner or later, distinct functional properties for the two A-type lamins will emerge.

Concluding Remarks

Since the discovery of protein isoprenylation and the recognition that prelamin A is prenylated, prevailing dogma has held that the carboxyl-terminal post-translational modifications must be critically important. Also, because lamin A biogenesis involved not only protein isoprenylation but three other processing steps, lamin A has generally been considered essential for the formation of the nuclear lamina. While miscellaneous studies in transfected cells have tended to support these views,^{15,44–46} no one had actually tested this dogma in vivo. With the development of an array of knock-in mouse models (Fig. 2), this situation has started to change. The study of several of these mice has already shown that lamin A and lamin C are functionally redundant in laboratory mice.^{33,42,48} Knock-in mouse experiments have also shown that exclusive production of nonfarnesylated prelamin A leads to cardiomyopathy, but not progeria.³³ Finally, we have learned that the direct synthesis of mature lamin A, bypassing prelamin A synthesis and processing, has no discernible adverse effects on either the targeting of lamin A to the nuclear envelope in vivo or mouse vitality.⁴⁸

While the absence of prelamin A processing appears to be inconsequential, defects in prelamin A processing wreak

havoc. Defects that block lamin A biogenesis and lead to an accumulation of farnesylated prelamin A cause severe progeroid syndromes. More work is required to understand the importance of the farnesyl lipid in disease pathogenesis. FTI treatment studies in HGPS mice have shown that blocking protein farnesylation has salutary effects; however, genetic studies with a “nonfarnesylated progerin mouse” have raised questions about whether protein prenylation is the only important factor in disease pathogenesis. Additional studies in mice, along with the ongoing clinical trials of FTI therapy in humans with HGPS,⁵² should help to clarify this issue.

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